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ORIGINAL ARTICLE / ORIGINALBEITRAG

Evaluation of Performance of Different Culture Media in *In Vitro* Shoot Propagation of Local Grape Varieties

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Abstract

Türkiye is one of the most important countries for vineyard areas and grape production. Viticulture in Türkiye, which has suitable cultivation opportunities, has an old and deep-rooted history and is also considered as the native land of the vine. Viticulture, which has been carried out for many years, has bred a great wealth of grape genotypes. The presence of local varieties plays an important role in this diversity. It is important to develop an effective *in vitro* tissue culture system for the preservation and reproduction of local gene resources. This study was carried out to determine the optimal nutrient media for micropropagation of local grape (*Vitis vinifera* L.) germplasm. For micropropagation, three different basal media were tested, Murashige and Skoog (MS), McCown and Lloyd's woody plant medium (WPM) and Nitsch and Nitsch (NN), all three combined with 1 mg L^{-1} BAP. The highest shoot rate (93.33%) was observed on explants cultured on 'Şire' grape variety NN media, whereas this parameter was reduced on explants cultured on WPM and MS media (86.67%). The highest shoot rate (93.33%) was observed on explants cultured on 'Elma' grape variety NN media, whereas this parameter was reduced on explants cultured on WPM and MS media (40.00 and 46.67%, respectively). Depending on the variety duration of bud break (%), shooting rate (%), number of leaves (pieces/plantlet) and shoot length (cm) parameters, MS, NN and WPM nutrient media did show statistical differences.

Keywords Viticulture · In vitro · Local · Optimization · Regeneration

Introduction

Türkiye has a fascinating viticultural potential due to its exceptional geographic situation in the northern hemisphere, and rich germplasm potential as the result of a great vine and wine cultural heritage. It is known that the vineyard areas of the countries located between 35–45 °C northern latitudes in the northern hemisphere are dense, and Türkiye is located in a geography suitable for viticulture since it is located between 36 and 42 °C northern latitudes (Uzun and Bayır 2008; Çelik 2012). Southeastern Anatolia Region (GAP) has an important share in terms of plant species diversity and productivity among our agricultural regions. The Southeastern Anatolia Region plays an important role in viticulture research in terms of the richness of genetic resources and the evaluation of grape varieties. The climate characteristics of Sanliurfa, one of the important provinces of the Southeastern Anatolia Region, are suitable for viticulture, the region's unique local varieties, and the increase of irrigable areas in the region with the Southeastern Anatolia Project are of great importance for the region's viticulture. However, due to the changes in the socio-cultural and ecological structure of the people in recent years, and the inability to keep up with modern cultivation, our local grape varieties, which have been well adapted to the region and have important characteristics for many years, are at risk of disappearing.

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Local varieties are valuable because they are highly adaptable to the ecological conditions they are in, they are resistant to diseases and pests, and they have many quality features such as taste and fruit characteristics. By using plant genetic materials, new genotypes can be developed that are resistant to many adverse conditions such as diseases and pests, salinity, drought, and existing biotic-abiotic stress factors, with new characteristics. Plant genetic resources are indispensable for meeting the increasing food demand and breeding new varieties that can adapt to the adverse conditions brought by global climate change (Sabır et al. 2018; Yılmaz et al. 2022).

The transfer of plant gene resources from the present to the future will be possible by protecting and preserving them. Advances in plant biotechnology have provided effective methods to conserve and restore plant diversity. *In vitro* conservation is especially playing an important role for vegetatively propagated and for non-orthodox seed plant species. The *in vitro* micropropagation technique, which is one of the tissue culture approaches, is also a preferred technique for the continuity and preservation of genetic resources. Local plant resources with high adaptability to adverse conditions will enable the reproduction and dissemination of pathogen-free material with high reproduction coefficient by micropropagation method *in vitro* conditions regardless of the season (Rajasekharan and Sahijram 2015). In addition, *in vitro* techniques offer a safe way to exchange plant material internationally, enable the creation of extensive collections using minimal space, allow the provision of valuable materials for wild population recovery, and facilitate molecular research and ecological studies (Tandon and Kumaria 2005; Cruz-Cruz et al. 2013).

The medium, which is one of the factors affecting *in vitro* micropropagation, plays an important and primary role in the effective reproduction of plant material. The basal salt formulation of a medium is a vital but often overlooked ingredient in many *in vitro* applications, as it regulates the growth and morphology of plant tissues by providing essential nutrients. Various media are used for micropropagation, such as MS, NN medium, B5 medium, and Gamborg medium. The choice of the medium is one of the important parameters for optimizing the regeneration protocol. Because the responses to the medium in the development of plant material different between species and even among varieties (Greenway et al. 2012).

This study was carried out to determine the optimal nutrient media for micropropagation of local grape (*Vitis vinifera* L.) germplasm. For micropropagation three different basal media were tested: MS, McCown and Lloyd's woody plant medium (WPM) and NN. *In vitro* micropropagation of the herbal materials used in the study has not been tried before.

For this reason, determining the most suitable nutrient medium for the nine regional varieties used in the study will play an important role in the preservation of the existence of the varieties, their reproduction and the acquisition of new genotypes with superior characteristics in breeding studies.

Materials and Methods

In the study, the samples were taken from 'Şire', 'Simore', 'Küllahi', 'Çiloreş', 'Hasani', 'Serpenekıran', 'Kızılbanki' and 'Heştur' varieties in the varieties collection plot at the Sanliurfa GAP Agricultural Research Institute Tektek station. Shoots were cultured as a node culture in the Harran University Agriculture Faculty Horticulture Department tissue culture laboratory. MS, NN, and WPM basal media treatments (Table 1) were tested using growth and other responses from various plant species.

Explants were taken during the active development period from grape varieties grown in the varieties collection plot and single-node micro-cuttings were prepared (Kara et al. 2022). The study was set up in a randomized plot factorial experiment design with 15 nodes in each plot with three replications. For surface sterilization (in a vertical air-

Table 1 The mineral concentrations of Murashige and Skoog (MS), Nitsch and Nitsch (NN) and McCown and Lloyd's woody plant medium (WPM) basal media

	Concentration in culture media (mg/l)		
Componentry	MS	NN	WPM
KNO_3	1900	950	0
NH_4NO_3	1650	720	400
$MgS0_4.7H_20$	370	185	370
$CaCl_2.2H_20$	440	220	96
$Ca(N0_3)_2.4H_20$	0	0	556
KH_2PO_4	170	68	170
$MnS0_4.4H_20$	22.3	25	22.3
K_2SO_4	0	0	990
KI	0.83	0	0
H_3BO_3	6.2	10	6.2
$ZnS0_4.7H_20$	8.6	10	8.6
$CuS0_4.5H_20$	0.025	0.025	0.25
$Na_2Mo04_2H_20$	0.25	0.25	0.25
$CoCI_2.6H_20$	0.025	0	0
$FeS0_4.7H_20$	27.8	27.8	27.8
Na_2EDTA	37.3	37.3	37.3
Nicotinic acid	0.5	5	0.5
Pyridoxin-HCl	0.5	0.5	0.5
Thiamin-HCl	0.1	0.5	1.0
Biotin	0	0.05	0
Folic acid	0	0.5	0
Myo-inositol	100	100	100
Glycine	2.0	2.0	2.0

flow sterile cabinet), the single-node micro-cuttings were soaked in 70% ethanol for 2 min and then in 12% sodium hypochlorite (NaOCl) solution for 15 min and then rinsed three times with sterile distilled water. After surface sterilization micro-cuttings were transferred to tubes of MS (Murashige and Skoog 1962), NN (Nitsch and Nitsch 1969) and WPM mediums (Lloyd and McCown 1980) containing 3% sucrose and 0.6% agar. A total of 1 mg L^{-1} BAP was added to the medium at the shoot formation stage. Plant preservative mixture (ppm) was added at a dose of 1 ml L^{-1} to prevent bacterial growth in explants. The explants in the culture medium were placed on shelves with a light source at 3000 lx m^{-2} illumination intensity in the climate room (25 ± 1 °C), developed in a 16/8 h light/dark photoperiod (Ekbiç and Yılmaz 2018; Şaşkın et al. 2022).

Recorded data were statistically analyzed by applying the analysis of variance technique (Gomez and Gomez 1984). The statistical difference of the mean values was detected utilizing LSD test ($p \leq 0.05$). Hierarchical clustering analysis (HCA) was conducted via the Software R (Version 4.1.1, R Foundation for Statistical Computing, Vienna, Austria).

Results and Discussion

Duration of Bud Break (%)

Nine grape varieties were transferred to three different basal environments in the study. Bud bursting started from the 5th day and continued until the 12th day. Data were collected three times based on the bud burst time (DBB) transfer date. Bud burst duration in cultivars differed in basal environments. Among the three basal environments tested in DBB1, NN 'Şire' and 'Hasani' grape varieties caused 46.67 and 40.00% bud burst, respectively, while no bud burst occurred in 'Simore', 'Çiloreş', 'Kızılbanki' and 'Heştur' grape varieties. When looking at DBB2, the highest bud burst time was observed in the MS (93.33%) and NN (86.67%) medium of the 'Şire' grape variety, respectively, while similar rates of bud burst were observed in the 'Şire'–WPM, 'Simore'–MS, 'Çiloreş'–MS and 'Hasani'–NN groups. The lowest bursting times were determined in MS and WPM applications of 'Heştur' grape variety. Considering DBB3, while it reached 100% in 'Şire'–MS application, the remaining varieties had bud burst of 20% or more in three basal medium applications (Fig. 1). Genotypic variability in *Vitis vinifera* L. cultivars cultured *in vitro* has been reported in previous studies (Galzy et al. 1990). The varying response of different cultivars to different basal environments may be due to differences in their nutrient composition (Table 1) (Barreto et al. 2006).

Shooting Rate (%)

In vitro micropropagation of local grape varieties was found to be important in variety–media interaction. The highest shooting rate was determined in 'Şire'–NN (93.33%) application, and the lowest shooting rate was determined as 'Heştur'–MS (20.00%) (Fig. 2). Contamination is a major problem in *in vitro* micropropagation and causes losses in shoot growth. One of the factors affecting shoot growth is the nutrient medium used. Chee and Pool (1987) reported that low KI and $MnSO_4$ concentrations in the medium were good for maximum shoot production in a study on inorganic media components developed for *in vitro* shoot propagation of *Vitis*. In this study, the spreading rate differed in different media applications due to contamination in some plants, genotype variability among grape varieties and the components that make up the content of the medium (Table 1) (Eftekhari et al. 2012).

Number of Leaves (Pieces/Plantlet)

In MS, NN and WPM applications, the number of shoot leaves for each cultivar was significant in cultivar and cultivar–media interaction. The highest number of leaves was determined in 'Heştur'–WPM (7.33) application, and the lowest number of leaves was determined in 'Hasani'–WPM (1.52) application. Considering the remaining cultivar–media applications, the number of leaves per plantlet was found to be 3.33 and above (Fig. 3). Mineral deficiency causes a significant decrease in the growth of explants grown *in vitro* and affects the average leaf number. While neglecting nitrate, phosphate and potassium significantly reduces the number of leaves, while it has been reported by studies that not giving calcium, sulfate, ammonium and magnesium has no significant effect (Ramage and Williams 2002; Kabylbekova et al. 2020). The presence of K_2SO_4 mineral in WPM basal medium supports the increase in the number of leaves in 'Heştur'–WPM treatment. Mineral concentrations vary according to basal environments. While nitrate plays an important role in the developmental process of plant, it is found in higher amounts in the MS environment than NN and WPM. The high amount of nitrate creates a toxic effect in some species and varieties and retards plant growth. In this study, it was determined that there was a difference in the response of grape varieties to basal environments, and the number of leaves increased in NN and WPM environments, which had less nitrate content than MS (Fig. 5).

Shoot Length (cm)

In MS, NN and WPM applications, shoot length for each cultivar was found to be important in cultivar and culti-

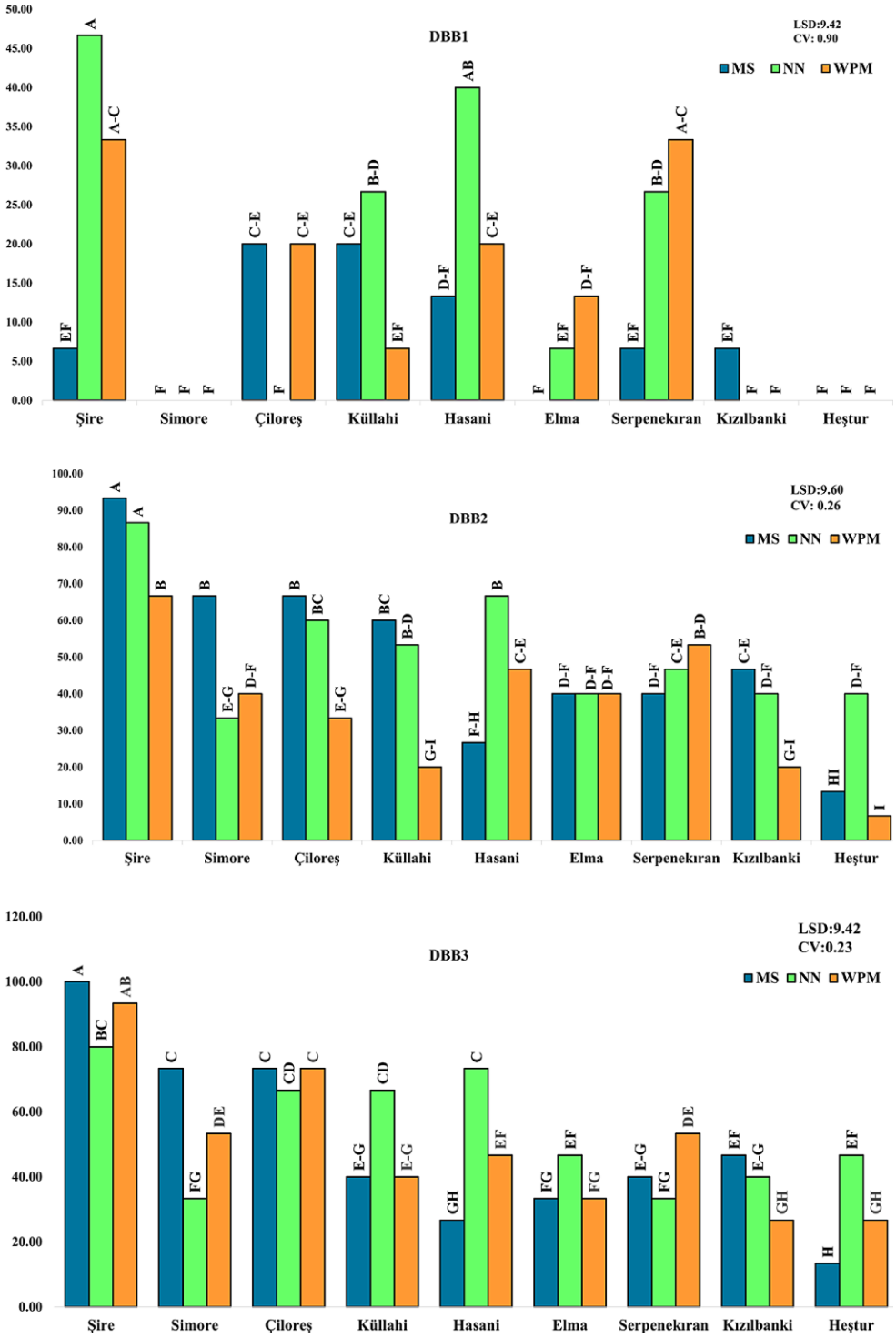


Fig. 1 Effects of three basal media on duration of bud break (*DBB*) of *Vitis vinifera* L. varieties. *MS* Murashige and Skoog, *WPM* Woody plant medium, *NN* Nitsch and Nitsch

var–media interaction. The highest shoot length was determined in 'Hasani'–NN (1.73) application, and the lowest shoot length was determined in 'Kullahi'–MS (0.47) application (Fig. 4). Although the differences in the stimulation of shoot elongation vary according to the species, the differences in the response of the plant material to the media components among the varieties may be related to the concentrations of the macronutrients. Galzy (1969) reported in a study that K and N concentrations are favorable for shoot development. MS medium is reported to be a suitable medium for plant regeneration due to high nitrogen levels in both nitrate and ammonium forms and relatively high ammonium nitrate ratio. However, because it contains high levels of ammonium ions, it may not be the most suitable

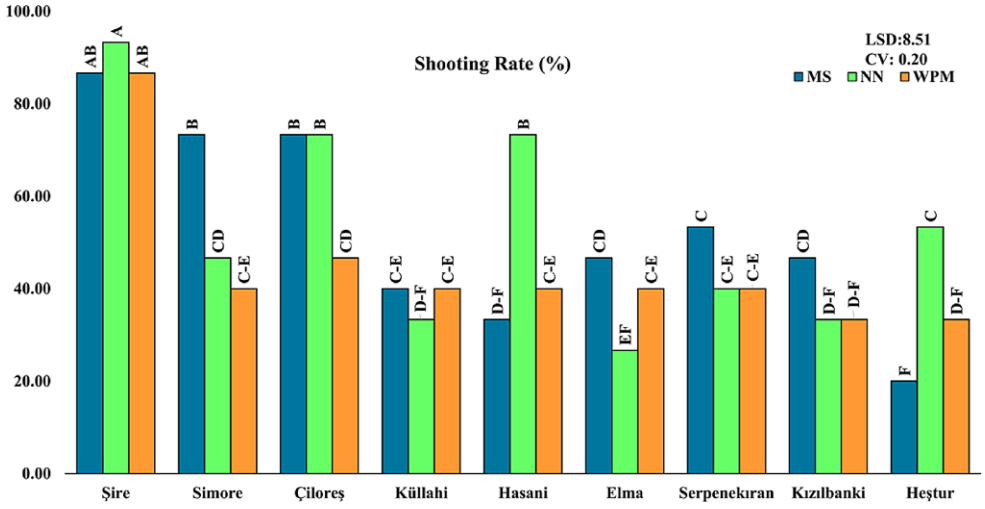


Fig. 2 Shooting rates (%) of varieties in Murashige and Skoog (*MS*), Nitsch and Nitsch (*NN*), and woody plant medium (*WPM*) basal medium

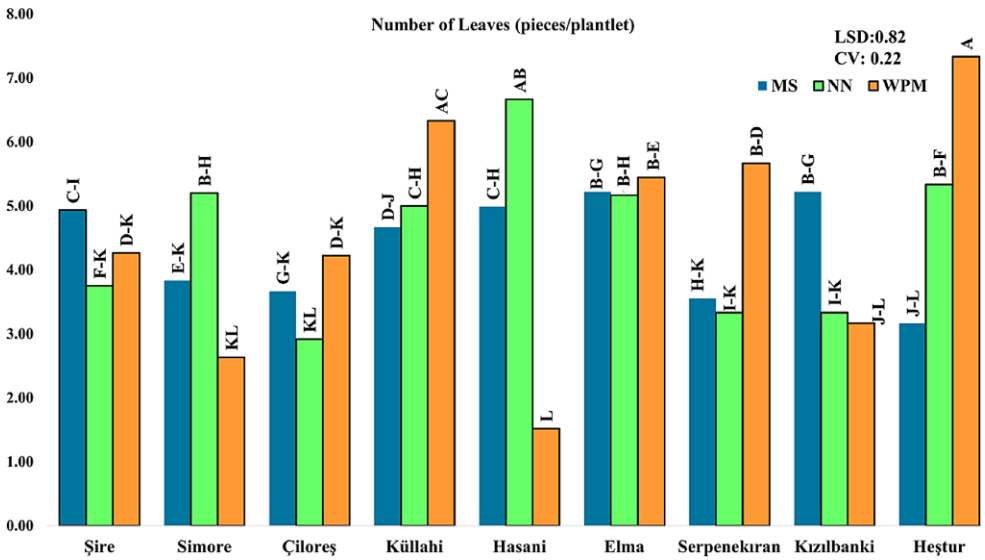
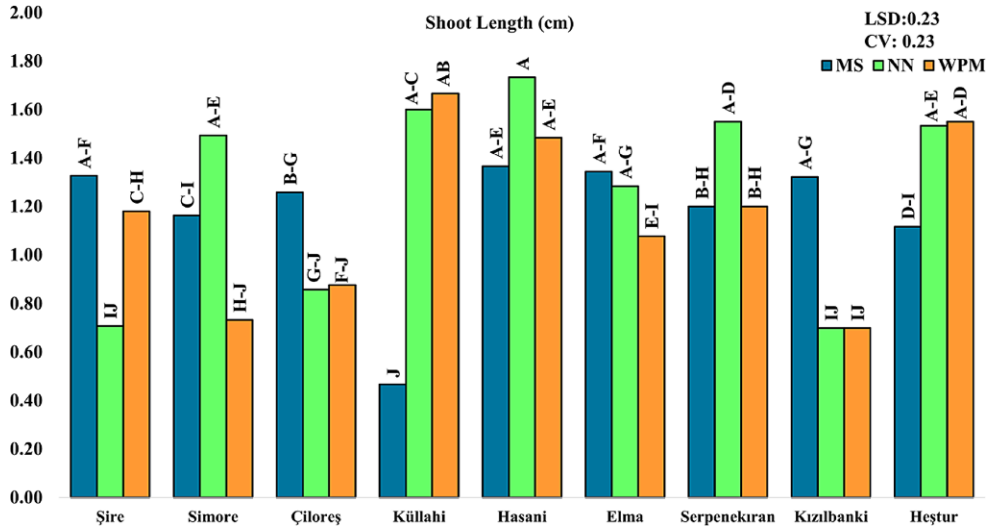


Fig. 3 Number leaves of varieties in Murashige and Skoog (*MS*), Nitsch and Nitsch (*NN*) and woody plant medium (*WPM*) basal medium

environment for optimum growth and regeneration for some plant species and cultivars. NN has about half as much total nitrogen as MS but has a similar ratio of ammonium nitrate. Similarly, WPM has less total nitrogen and less ammonium than MS (Gamborg et al. 1976; Phillips and Garda 2019). In this study, it was determined that the majority of grape varieties showed better growth in shoot length in NN and WPM medium compared to MS (Fig. 5).

Hierarchical Clustering Analysis (HCA)

Morphological responses of local grape varieties in different basal media were characterized by HCA. In HCA, two-way dendrograms were generated that clustered the variables (items and samples) separately and are represented as heatmaps, where red and blue symbolize varieties (shown in rows) with high and low corresponding morphology parameters (shown in column), respectively (Fig. 6). HCA was performed on six different parameters, including morphological (Fig. 6). A heatmap graph of the HCA analysis shows that the treatments were divided into four different clusters (Fig. 6). 'Elma' (MS, NN, WPM), 'Hasani' (MS, WPM), 'Kızılbanki' (MS, NN, WPM), 'Heştur' (MS, NN), 'Serpenekıran' (MS, NN), 'Simore' (NN, WPM), 'Çiloreş'–WPM and 'Küllahi'–MS were in group I; 'Heştur'–WPM and 'Küllahi'–WPM were in group II; 'Şire' (MS, NN, WPM), 'Çiloreş' (MS, NN), 'Simore'–MS were in group III and 'Hasani'–NN, 'Küllahi'–NN and 'Serpenekıran'–WPM were clustered in group IV. Based on HCA, morphological parameters were grouped into four groups. Leaf number and Shoot length were in group A and B. Leaf number showed the highest value in local grape varieties 'Heştur'–WPM under different nutrient media conditions, and these value decreased with the effect of NN and MS. Shoot length showed the highest value in 'Hasani'–NN under different nutrient



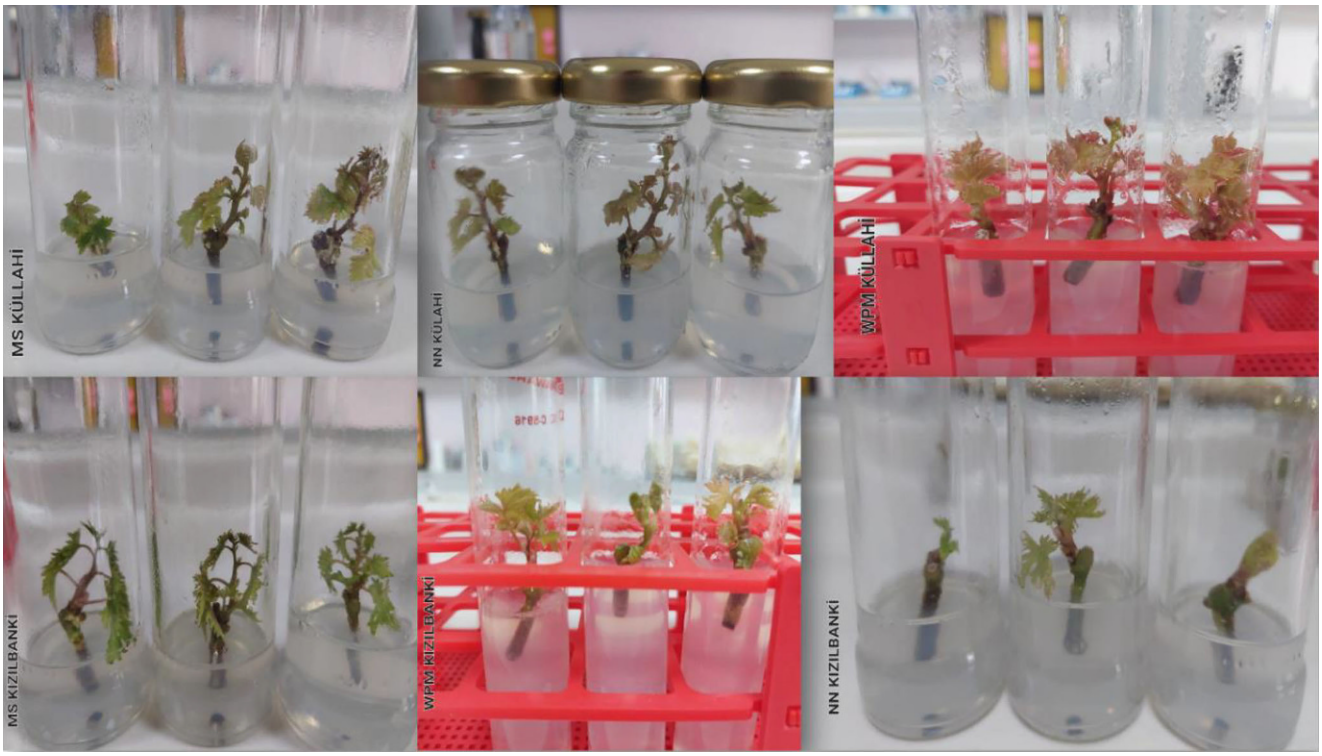


Fig. 4 Shoot length of varieties in Murashige and Skoog (*MS*), Nitsch and Nitsch (*NN*) and woody plant medium (*WPM*) basal medium

Fig. 5 The appearance of 'Küllahi' and 'Kızılbanki' cultivars in Murashige and Skoog (*MS*), Nitsch and Nitsch (*NN*) and woody plant medium (*WPM*) mediums

media conditions, and these values decreased with the medias WPM and MS. Shoot length decreased in cultivar-application groups clustered in I and III. Duration of bud break-1 were placed into group C. Duration of bud break-2 and Duration of bud break-3 and shooting rate in leaves were in group D. Duration of bud break-1 showed the highest value respectively in 'Şire'–NN, 'Hasani'–NN and 'Serpenekıran'–WPM. This parameter data showed a decrease in the remaining variety–media applications. Shooting rate was the highest in 'Şire'–NN; the highest duration of bud break-2 and duration of bud break-3 'Şire'–MS were in local grape variety. HCA analysis, 'Kızılbanki'–NN, 'Kızılbanki'–WPM, 'Serpenekıran'–MS, 'Küllahi'–MS, 'Heştur'–MS, 'Simore'–WPM, 'Hasani'–WPM applications and 'Hasani'–NN, 'Küllahi'–NN, 'Şire'(NN, MS, WPM) applications showed a negative correlation. 'Şire' (MS, WPM), 'Serpenekıran'–WPM and 'Hasani'–NN showed positive correlations in shooting rate, DBB2 and DBB3 parameters in variety–media applications. The varying responses of different genotypes to different basal

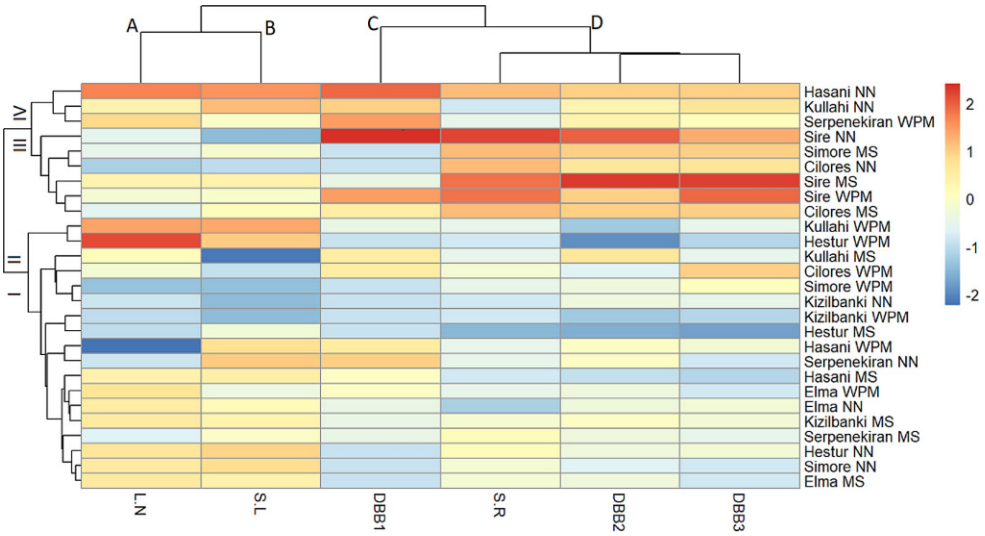


Fig. 6 Hierarchical clustering analysis for morphological parameters in nine grape cultivars. *DBB* Duration of bud break, *S.R* Shooting rate, *S.L* Shoot length, *L.N* Leaf Number. *I*, *II*, *III* and *IV* represent groups of studied treatments. *A*, *B*, *C* and *D* represent the studied parameter groups

mediums may be due to differences in nutrient composition. For example, the amount of $CaCl_2$ is higher in MS than in WPM and NN. Similarly, KI is not found in WPM and NN, while it is present in MS. For example, in the study on *in vitro* shoot proliferation of *V. rotundifolia*, it was stated that MS and ½ MS nutrient medium had the same effect, while dwarf shoots were obtained from WPM nutrient medium (Gray and Benton 1991). Mhatre et al. (2000) used 'Thompson Seedless', 'Sonaka' and 'Tas-e Ganesh' grape cultivars at different stages of *in vitro* propagation of different modified forms of MS, NN and WPM. Genotypic variability in *Vitis vinifera* L. cultivars cultured *in vitro* by studies has been previously reported (Harris and Stevenson 1982; Galzy et al. 1990; Yıldırım and Özdemir 2018). In this study, the morphological responses of nine local grape varieties to three basal environments differed.

Conclusion

The importance of genetic resources in the world and in our country is being understood better day by day. In fact, developments show that the most important natural resource of the current century is genetic resources. For this reason, grape genetic resources should be protected as well as all our other natural resources. It is of great importance to ensure that these resources are used in a beneficial way. The way to achieve this can be listed as protecting, defining, evaluating, making use of diversity and using it primarily for breeding purposes. As a result of natural selection, the efforts to protect local varieties and types with some good characteristics, before they are destroyed, should be accelerated. One of the ways to protect local varieties is to reproduce and preserve them with tissue culture, which is a biotechnological method. The aim of this study was to reproduce and protect local grape varieties, which are about to disappear, by tissue culture method. Basal media compatibility was tested for each cultivar for sustainable *in vitro* propagation. The medium formulation experiment showed strong effect on shoot proliferation and growth parameters when three different media were tested (MS, WPM and NN).

Author Contribution H. Ekinci designed the research and conducted the review and editing; İ. Rastgeldi, N.Şaşkın, B.E. Ak, Ş. Korkmaz assisted with the project and resources.

Conflict of interest H. Ekinci, İ. Rastgeldi, N. Şaşkın, B.E. Ak and Ş. Korkmaz declare that they have no competing interests.

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